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10/591,407	12/08/2006	Takumi Teratani	701053	2167

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EXAMINER	
NGUYEN, QUANG	

ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
03/24/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/591,407	TERATANI ET AL.	
	Examiner	Art Unit	
	QUANG NGUYEN, Ph.D.	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 November 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *8-13 and 35* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *8-13 and 35* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment filed on 11/16/2009 was entered.

Amended claims 8-13 and new claim 35 are pending in the present application.

Response to Amendment

The rejection under 35 U.S.C. 102(b) as being anticipated by Vassilieva et al. (Experimental Cell Research 258:361-373, 2000; IDS).\ was withdrawn in light of Applicant's amendment.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Amended claims 8, 10, 12 and new claim 35 are rejected under 35 U.S.C. 102(b) as being anticipated by Loring, J. (WO 99/27076; IDS). ***This is a modified rejection necessitated by Applicant's amendment.***

Loring already disclosed at least a method for obtaining non-mouse embryonic stem cells, including rat embryonic stem cells, said method comprises: (a) culturing harvested blastocysts or delayed-blastocysts in individual wells of a 24-well plate in any appropriate medium under any conditions which allow for growth and proliferation of ES cells, with an exemplified medium is DMEM with glutamine and high glucose

supplemented with 15% fetal bovine serum, 1 X non-essential amino acids, 0.1 mM 2-mercaptoethanol and antibiotics; (b) the inner cell mass (ICM) was removed under conditions that minimize contamination with other cell types after about 3 to 5 days in culture using a micropipette and then dissociated with 0.25% trypsin, under which conditions the ICM is dispersed either to a single cell suspension or **more preferably to produce small groups of cells**; (c) ICM cultures were cultured in 6 well dishes and **colonies arising from the dispersed ICMs will be selected by morphology criteria** with exogenous growth factors (for example, bFGF, LIF and SCF) alone or in combination may be added to the cultures **or** ICM cultures were cultured on any feeder layer that is not necessarily limited to SNL 76/7 cells producing LIF, such as STO cells; (d) after about a week of culture, colonies that resembled ES cells were dissociated and sub-cultured; (e) the sub-cultured ES cells were passed once to obtain cell lines BNRB-1 and FRDB-1 which are AP positive; and (e) the rat cell lines were co-cultured with mouse ES cells to obtain pluripotent rat ES cells which differentiated into a number of morphologically different cell types and embryoid bodies, including the ability of making a transgenic rat (see at least the abstract; pages 11-14; particular example 2, page 26, lines 7-8; examples 4-6). Loring also disclose specifically that the co-culture method arises from the observations that mouse ES cells differentiate more easily and often when they were cultured at low density rather than high density (page 13, lines 24-27).

It is noted that in step (a), the exemplified medium for culturing harvested blastocysts or delayed-blastocysts does not contain any LIF; and therefore this teaching meets the limitation of step (A) of amended claim 8. Moreover, Loring also stated that

"Culture medium (LIF; LIF and SCF, or LIF, SCF, and bFGF) made no apparent difference in the early blastocyst culture" (page 24, lines 15-16). Additionally, since the term **"substantially serum free" is defined in the present application to mean not to contain serum in an amount that rat ES cell loses the properties as an ES cell** (e.g., becomes negative for alkaline phosphatase activity) due to the effect of serum (page 17, lines 18-22); and Loring does not teach the use of any culture medium with rat LIF; along with the open language of the term "comprises" for the claimed method, the above teachings of Loring, J. meet every limitation of the claims as written.

Therefore, the reference anticipates the instant claims.

Response to Arguments

Applicants' argument related to the above rejection in the Amendment filed on 11/16/09 (pages 5-6) has been fully considered but it is respectfully not found persuasive.

Applicants argue basically that a high concentration of LIF is used for the formation of rat inner cell masses in Loring (100-200 units/ml of LIF); and therefore the reference does not anticipate the instant claims.

Loring disclosed a method for obtaining non-mouse embryonic stem cells, including rat embryonic stem cells, said method comprises the step of culturing harvested blastocysts or delayed-blastocysts in individual wells of a 24-well plate in any appropriate medium under any conditions which allow for growth and proliferation of ES cells, with an exemplified medium is DMEM with glutamine and high glucose

supplemented with 15% fetal bovine serum, 1 X non-essential amino acids, 0.1 mM 2-mercaptoethanol and antibiotics (page 12, lines 18-22). Therefore this teaching meets the limitation of step (A) of amended claim 8. Moreover, Loring also stated that "Culture medium (LIF; LIF and SCF, or LIF, SCF, and bFGF) made no apparent difference in the early blastocyst culture" (page 24, lines 15-16). Additionally, please also note that Loring taught specifically that a ICM cultures were cultured on any feeder layer that is not necessarily limited to SNL 76/7 cells producing LIF, such as STO cells which are not genetically engineered to produce LIF. Furthermore, at the effective filing date of the present application, JP 05-304951 (also see provided English translation, IDS) disclosed the use of a culture broth comprises IGF-II **OR** IGF-II and LIF for establishing ES/EC cells, including rat ES cells (see translation at page 12, paragraph 16); and stated that "From theses results of comparative cultivation, it was found that IFG-II is essential for the maintenance and growth of ES cells" (see translation at page 17, last sentence of second last paragraph).

Accordingly, the Loring reference anticipates the instant claims as broadly written.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Amended claims 8 and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Loring, J. (WO 99/27076; IDS) in view of Takahama et al. (Oncogene 16:3189-3196, 1998; IDS). ***This is a modified rejection necessitated by Applicant's amendment.***

Loring already disclosed at least a method for obtaining non-mouse embryonic stem cells, including rat embryonic stem cells, said method comprises: (a) culturing harvested blastocysts or delayed-blastocysts in individual wells of a 24-well plate in any appropriate medium under any conditions which allow for growth and proliferation of ES cells, with an exemplified medium is DMEM with glutamine and high glucose supplemented with 15% fetal bovine serum, 1 X non-essential amino acids, 0.1 mM 2-mercaptoethanol and antibiotics; (b) the inner cell mass (ICM) was removed under conditions that minimize contamination with other cell types after about 3 to 5 days in culture using a micropipette and then dissociated with 0.25% trypsin, under which conditions the ICM is dispersed either to a single cell suspension or **more preferably to**

produce small groups of cells; (c) ICM cultures were cultured in 6 well dishes and **colonies arising from the dispersed ICMs will be selected by morphology criteria** with exogenous growth factors (for example, bFGF, LIF and SCF) alone or in combination may be added to the cultures **or** ICM cultures were cultured on any feeder layer that is not necessarily limited to SNL 76/7 cells producing LIF, such as STO cells; (d) after about a week of culture, colonies that resembled ES cells were dissociated and sub-cultured; (e) the sub-cultured ES cells were passed once to obtain cell lines BNRB-1 and FRDB-1 which are AP positive; and (e) the rat cell lines were co-cultured with mouse ES cells to obtain pluripotent rat ES cells which differentiated into a number of morphologically different cell types and embryoid bodies, including the ability of making a transgenic rat (see at least the abstract; pages 11-14; particular example 2, page 26, lines 7-8; examples 4-6). Loring also disclose specifically that the co-culture method arises from the observations that mouse ES cells differentiate more easily and often when they were cultured at low density rather than high density (page 13, lines 24-27). It is noted that in step (a), the exemplified medium for culturing harvested blastocysts or delayed-blastocysts does not contain any LIF; and therefore this teaching meets the limitation of step (A) of amended claim 8. Moreover, Loring also stated that "Culture medium (LIF; LIF and SCF, or LIF, SCF, and bFGF) made no apparent difference in the early blastocyst culture" (page 24, lines 15-16).

Loring does not teach specifically the use of rat LIF-containing culture medium in steps (C)-(E).

At the effective filing date of the present application, Takahama et al already cloned cDNA encoding a rat LIF and demonstrated that **culture supernatant of the rat LIF cDNA-transduced rat fibroblast cell line could maintain the stem-cell phenotype of rat ES cells which showed alkaline phosphatase activity, and this effect was much stronger than that by murine LIF** (see at least the abstract). Takahama et al specifically taught that the availability of rat LIF cDNA will assist the establishment of in vitro culture conditions of rat ES cells and maintaining these cells in an undifferentiated state (page 319, col. 1).

It would have been obvious for an ordinary skilled artisan to modify the method of Loring by also selecting and using specifically rat LIF in a culture medium for culturing primary rat embryonic stem cells obtained from a culture of dissociated inner cell mass, and subsequent sub-culture steps for establishment of rat embryonic stem cells in light of the teachings of Takhama et al.

An ordinary skilled artisan would have been motivated to carry out the above modification because rat LIF has been shown by Takahama et al to be effective in maintaining the stem cell phenotype of rat ES cells which showed alkaline phosphatase activity, and its effect is much stronger than that of murine LIF.

An ordinary skilled artisan would have a reasonable expectation of success in light of the teachings of Loring together with Takahama et al., coupled with a high level of skill of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Amended claims 8-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Loring, J. (WO 99/27076; IDS) in view of Price et al. (WO 98/30679; IDS). ***This is a modified rejection necessitated by Applicant's amendment.***

The teachings of Loring were presented above. However, Loring does not teach specifically the use of a culture medium comprising a serum replacement reagent.

At the effective filing date of the present application, Price et al already taught **the use of a serum replacement medium to support the growth of embryonic stem cells in culture to avoid many problems associated with the use of serum as well as time consuming pre-screening process of serum** (see at least the abstract; Summary of the Invention, page 3, second and third paragraph; and examples).

It would have been obvious for an ordinary skilled artisan to modify the method of Loring by also using a serum replacement medium for establishment of pluripotent rat embryonic stem cells in light of the teachings of Price et al.

An ordinary skilled artisan would have been motivated to carry out the above modification because the use of a serum replacement medium to support the growth of embryonic stem cells in culture avoids many problems associated with the use of serum as well as time consuming pre-screening process of serum as taught by Price et al.

An ordinary skilled artisan would have a reasonable expectation of success in light of the teachings of Loring together with Price et al., coupled with a high level of skill of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Amended claims 8 and 11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Loring, J. (WO 99/27076; IDS) in view of Vassilieva et al. (Experimental Cell Research 258:361-373, 2000; IDS) and Mandalam et al (US 7,297,539). ***This is a new ground of rejection necessitated by Applicant's amendment.***

The teachings of Loring were presented above. However, Loring does not teach specifically the step of mechanically dissociating the primary embryonic stem cells for passaging.

At the effective filing date of the present application, in a method for establishment of SSEA-1- and Oct-4-expressing rat embryonic stem-like cell lines, Vassilieva et al already taught selecting and passaging colonies representing typical morphology of compacted ES cells were selected and passaged every day by mechanical disaggregation (see at least the abstract and particularly the section "Establishment of rat ES-like cell lines" on page 362 and col. 1 of page 363 and Table 1).

Additionally, at least in a method for growing human embryonic stem cells Mandalam et al also taught selecting individual ES cell colony by micropipette and/or ES cells are triturated with a pipette into clumps of adherent cells, about 10-2000 cells in size, which were then passaged into the new culture environment (see at least

Summary of the Invention; and particularly col. 9, line 53 continues to line 3 of col. 10 and lines 48-63 in col. 10).

It would have been obvious for an ordinary skilled artisan to modify the method of Loring by also mechanically dissociating the primary embryonic stem cells for passaging in light of the teachings of Vassilieva et al and Mandalam et al.

An ordinary skilled artisan would have been motivated to carry out the above modification because mechanically dissociating ES cells during cell passage has been taught successfully in the preparation of rat and human ES stem cells by Vassilieva et al and Mandalam et al, respectively.

An ordinary skilled artisan would have a reasonable expectation of success in light of the teachings of Loring, Vassilieva et al and Mandalam et al., coupled with a high level of skill of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments related to the above obviousness rejections in the Amendment filed on 11/16/09 (pages 6-8); along with the 1.132 Declaration of Takahiro Ochiya dated 11/16/09, have been fully considered but they are respectfully not found persuasive for the reasons discussed below.

Applicants argue that Loring does not disclose culturing a rat blastocyst in a LIF-free culture medium to form an inner cell mass; and that it was commonly held belief that LIF must be added to the medium to form an inner cell mass from a blastocyst and the inventors discovered that the rat inner cell masses can be efficiently formed by culturing rat blastocysts in a LIF-free medium. The 1.132 Declaration of Takahiro Ochiya described that the use of a LIF-free medium results in the formation of more inner cell masses than when a LIF-containing medium is used. Applicants further argue that rat ES cells produced by the inventive method demonstrate superior effects when compared to the cells of Loring, for example the BNRB-1 cells derived from rat embryo of Loring can not form embryoid bodies before the co-culture with mouse ES cells; while rat ES cells obtained by the inventive method are capable of forming embryoid bodies even though the method does not comprise the step for co-culturing with non-rat ES cells. Furthermore, the rat pluripotent cells obtained by the co-culture with mouse ES cells are not recognized by the art as true rat ES cells since their chimeric rat-producing ability has not been confirmed.

Firstly, once again Loring disclosed a method for obtaining non-mouse embryonic stem cells, including rat embryonic stem cells, said method comprises the step of culturing harvested blastocysts or delayed-blastocysts in individual wells of a 24-well plate in any appropriate medium under any conditions which allow for growth and proliferation of ES cells, with an exemplified medium is DMEM with glutamine and high glucose supplemented with 15% fetal bovine serum, 1 X non-essential amino acids, 0.1 mM 2-mercaptoethanol and antibiotics (page 12, lines 18-22). Therefore this teaching

meets the limitation of step (A) of amended claim 8. Moreover, Loring also stated that "Culture medium (LIF; LIF and SCF, or LIF, SCF, and bFGF) made no apparent difference in the early blastocyst culture" (page 24, lines 15-16). Additionally, please also note that Loring taught specifically that ICM cultures were cultured on any feeder layer that is not necessarily limited to SNL 76/7 cells producing LIF, such as STO cells which are not genetically engineered to produce LIF. Furthermore, at the effective filing date of the present application, JP 05-304951 (also see provided English translation, IDS) disclosed the use of a culture broth comprises IGF-II **OR** IGF-II and LIF for establishing ES/EC cells, including rat ES cells (see translation at page 12, paragraph 16); and stated that "From theses results of comparative cultivation, it was found that IFG-II is essential for the maintenance and growth of ES cells" (see translation at page 17, last sentence of second last paragraph).

Secondly, the higher efficiency of forming inner cell masses in the absence of LIF as described in the 1.132 Declaration of Takahiro Ochiya is irrelevant because as already noted above Loring already taught the step of culturing harvested blastocysts or delayed-blastocysts in individual wells of a 24-well plate in any appropriate medium under any conditions which allow for growth and proliferation of ES cells, with an exemplified medium is DMEM with glutamine and high glucose supplemented with 15% fetal bovine serum, 1 X non-essential amino acids, 0.1 mM 2-mercaptoethanol and antibiotics (page 12, lines 18-22); or alternatively ICM cultures were cultured on any feeder layer that is not necessarily limited to SNL 76/7 cells producing LIF, such as STO cells which are not genetically engineered to produce LIF.

Thirdly, please note that the instant claims are directed to a method for producing a rat embryonic stem cell; and as broadly written the claims encompass and do not necessarily exclude co-culturing rat embryonic stem cells in the presence of mouse embryonic stem cells.

Fourthly, Loring taught explicitly that rat ES cells were obtained only after co-culturing with mouse ES cells; and the co-cultured mouse ES cells were killed after HAT treatment and the surviving rat ES cells were obtained and isolated (see at least page 26).

Fifthly, it is noted that Ueda et al (PLos One, Volume 3, Issue 7, e2800, pages 1-9, 2008) disclosed a method for establishment of rat embryonic stem cells and making of chimera rats. **The authors of this post-filing article includes the names of both inventors of the present application (Takumi Teratani and Takahiro Ochiya)**. **Although the disclosed method for establishment and maintenance of rat ES cell lines is the same as that of the present application**, and the reported established rat embryonic stem cells are **substantial similar** to rat embryonic stem cells of the present application, it is noted that for some unknown reasons **unlike the rat embryonic stem cells described by the present application as well as known human ES and murine ES cells, rat embryonic stem cells of Ueda et al are negative for alkaline phosphatase** (page 5, col. 1, third paragraph). With respect to the rat embryonic stem cells of Ueda et al, Buehr et al (Cell 135:1287-1298, 2008) stated "A recent report claims derivation of rat ES cells in conventional ES cell cultures with feeders, LIF, and serum (Ueda et al., 2008). However, consistent with many previous reports **we find**

that these conditions are not adequate to drive or maintain rat ES cells. Furthermore, the cell-surface and marker profile reported by Ueda and colleagues are inconsistent with ES cell identity and suggest that their cells may be EpiSC like" (page 1295, col. 1, last line continues to first paragraph of col. 2). Li et al. (Cell Research 19:173-186, 2009) disclosed derivation and transcriptional profiling analysis of pluripotent stem cell lines from rat blastocysts. Li et al also stated **various short-comings of rat ES cell lines reported by Ueda et al** (see page 163, col. 2, last paragraph continues to first paragraph of col. 1 on page 194).

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of

the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (571) 272-0776.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's SPE, Joseph T. Woitach, Ph.D., may be reached at (571) 272-0739.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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/QUANG NGUYEN/
Primary Examiner, Art Unit 1633